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AN AUTORADIOGRAPHIC STUDY OF THE TRANSLOCATION OF ^{14}C-LABELLED ASSIMILATES IN *ERAGROSTIS CURVULA* (SCHRAD.) NEES AT DIFFERENT STAGES OF VEGETATIVE GROWTH

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Abstract

Autoradiography was used to study the movement of ^{14}C-labelled assimilates in *Eragrostis curvula* at several stages of vegetative growth. The assimilates were labelled by allowing single leaves of plants to photosynthesize in an atmosphere containing radioactive carbon-dioxide ($^{14}CO_2$). Leaves of different ages, located on different tillers, were treated.

Assimilates were exported mainly to growing regions, such as young developing leaves, and to the roots. The pattern of distribution of assimilates changed during the growth of the plant. During the early stages of development (seedling to four-tiller stage) there was extensive intertiller translocation between the main and daughter tillers and vice versa. During the later stages of growth (six-tiller to mature vegetative stage) intertiller translocation decreased considerably and tillers became more independent.

Uittreksel

'N OUTORADIOGRAFIESE STUDIE VAN DIE TRANSLOKASIE VAN ^{14}C-GEMERKTE ASSIMILATE IN *ERAGROSTIS CURVULA* (SCHRAD.) NEES BY VERSKILLENDE VEGETATIEWE GROEISTADIA.

Outoradiografie is gebruik om die beweging van ^{14}C-gemerkte assimilate in *Eragrostis curvula* by verskillende vegetatiewe groeistadia te bepaal. Assimilate is gemerk deur enkele blare van plante te laat fotositetiseer in 'n atmosfeer wat radio-aktiewe koolstofdioksied ($^{14}CO_2$) bevat. Blare van verskillende ouderdomme, en op verskillende lote is so behandel.

Assimilate is hoofsaaklik vervoer na groeiende dele soos jong, ontwikkelende blare, en na die wortels. Die distribusiepatroon van assimilate het verander terwyl die plant gegroei het. Gedurende die vroeë groeistadia (kiemplant tot 4-loot stadium) was daar uitgebreide interloot-translokasie tussen die hoof- en dogter-lote en omgekeerd. Gedurende latere groeistadia (6-loot tot volwasse vegetatiewe stadium) het interloot-translokasie aansienlik afgeneem, en het lote meer onafhanklik geword.

Introduction

The translocation of ^{14}C-labelled assimilates in members of the Gramineae has been the subject of several investigations. Well-defined distribution patterns of assimilates have been found in cereals, such as wheat (Quinlan and Sagar, 1962; Lupton, 1966) and in grasses such as *Phleum pratense* L (Williams, 1964) and *Lolium multiflorum* Lam. (Sagar and Marshall, 1966; Marshall, 1967). These studies have shown how the various parts of a graminaceous plant are interrelated in terms of assimilate movement. The object of the present study was to contribute to work done in this direction since little is known about the distribution patterns of photosynthetic assimilates in grasses indigenous to

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South Africa. *Eragrostis curvula* was chosen because it is not only a common veld grass but is also important agronomically.

MATERIAL AND METHODS

Cultural conditions:

Seeds of *Eragrostis curvula* (Ermelo cultivar) were sown in 15 cm clay pots containing a 1:1 mixture of sand and soil. The sand and soil were autoclaved at a pressure of 1 bar for 15 min prior to filling the pots. The plants were grown in a temperature-controlled glasshouse which was provided with two air-conditioning units. This ensured that the plants were not exposed to very high or low temperatures. Natural daylight was extended by six 200 watt mercury-tungsten lamps to give a 16h photoperiod. Watering was carried out as frequently as necessary to prevent any moisture shortage. Small applications of a commercial fertilizer were made uniformly to each pot.

Under these conditions plants were healthy and grew vigorously. Seedlings were selected for uniformity and thinned to four per pot. When most plants had two tillers, selection for uniform development was again carried out, and the plants were thinned to one per pot.

Assimilation of $^{14}CO_2$

The method used to introduce labelled carbon into plants was based on a technique similar to that of Quinlan and Sagar (1962). Single leaves of plants were allowed to photosynthesize in an atmosphere containing $^{14}CO_2$. Radioactive carbon-dioxide was produced by the addition of excess 50 per cent lactic acid to an aqueous solution of ^{14}C-labelled sodium carbonate ($Na_2{}^{14}CO_3$). The specific activity of the sodium carbonate was 10 mc/mM.

In preliminary experiments it was found that a good autoradiographic image could be produced if small plants (seedling to six-tiller stage) received a dose of 0,5 μc of radioactivity and bigger plants (eight-tiller to mature vegetative stage) 1 μc. A larger dose was required for larger plants because of dilution of the tracer within the plant.

The administration of $^{14}CO_2$ was performed in the glasshouse in bright sunlight on a clear day. The assimilation period lasted for 6h, from 0930 to 1530h. The plants were left in the glasshouse and harvested 18h later.

Autoradiography:

Soil was carefully washed free from the roots and the plants were freeze-dried and autoradiographed following a technique modified from Yamaguchi and Crafts (1958), Little (1962) and Crafts and Yamaguchi (1964).

Mounted plants were exposed to Kodak Blue Brand X-ray film for two weeks if the radioactive dose was 0,5 μc or for 1 week if 1 μc of radio-activity was given. The manufacturer's instructions for processing X-ray films were followed.

Treatments:

Treatments were carried out at seven different stages spanning the development of the plant. These stages together with the leaves which were treated are shown in Table 1.

TABLE 1.

Leaves which assimilated $^{14}Co_2$ in plants at different stages of development.

STAGE OF DEVELOPMENT	TREATMENT LEAVES			
(i) Seedling	Youngest fully expanded leaf on main tiller	Oldest green leaf on main tiller	—	—
(ii) 1-Tiller				
(iii) 2-Tiller			Youngest fully expanded leaf on oldest daughter tiller	Youngest fully expanded leaf on youngest daughter tiller
(iv) 4-Tiller				
(v) 6-Tiller				—
(vi) 8-Tiller				as above
(vii) Mature Vegetative				—

In this investigation, the primary or dominant tiller was referred to as the main tiller and the secondary or subsidiary tillers were called daughter tillers. A diagrammatic representation of a plant at the four-tiller stage is illustrated in Fig. 1. The main tiller (MT) is shown, together with the four daughter tillers (T_1, T_2, T_3, T_4) at different stages of development (T_1, oldest and T_4 youngest). The youngest fully expanded leaf (indicated by numeral I) on the main tiller and on the oldest and youngest daughter tillers, is shown. A fully expanded leaf was recognized by a clearly visible ligule. In addition, the oldest green leaf on the main tiller (II) is shown.

Each plant was treated on a single leaf, different plants being used to determine the patterns of distribution of labelled assimilates from the different leaves. Uniform plants were selected for treatment. Each treatment was replicated twice.

RESULTS

The results are presented as visual assessments of autoradiographs. Photographs of mounted plants and their corresponding autoradiographs are shown. Since there was very close agreement between replicates in the majority of

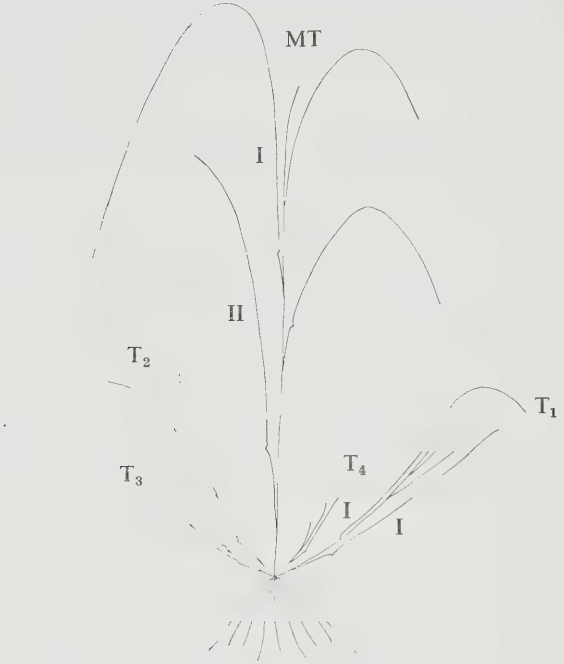


FIG. 1.
Diagrammatic representation of a plant at the four-tiller stage of development.

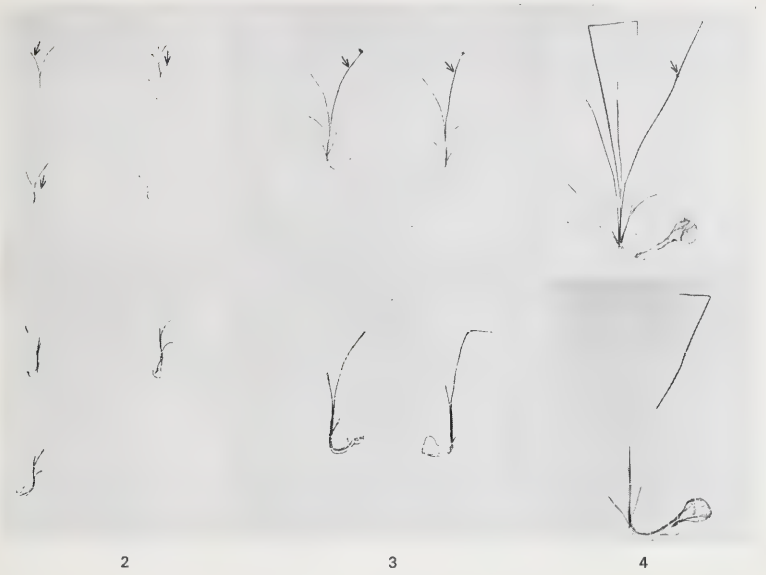
treatments, only one replicate set, unless otherwise stated, is presented. In the photographs of the mounted plants, the treated leaf is indicated by an arrow.

The patterns of distribution of labelled assimilates during the various vegetative stages of growth, following assimilation of $^{14}CO_2$ by single leaves of different ages located on either the main or daughter tillers are shown in Figs. 2–13.

(a) *Treatment of youngest fully expanded leaf on main tiller:*

In the seedling stage (Fig. 2, plant at upper left hand corner), ^{14}C-assimilates from the treated leaf moved mainly to those parts which were actively growing, accumulating in the young developing leaf, in the lower part of the expanding leaf, in the roots and to a lesser extent in the mature leaf.

An unlabelled plant, at the lower right hand corner of Fig. 2, was treated in exactly the same way as the other three labelled plants. As can be seen, it did



Figs. 2–4

Photographs of mounted plants above, autoradiographs below. Fig. 2. Treatments of plants at the seedling stage: Youngest fully expanded leaf treated (plant at upper left hand corner); oldest green leaf treated (plants at upper right and lower left hand corners); and an untreated plant (lower right hand corner). Fig. 3. Youngest fully expanded leaf on main tiller of a duplicate set of plants at the one-tiller stage treated. Fig. 4. Youngest fully expanded leaf on main tiller of a plant at the two-tiller stage treated.

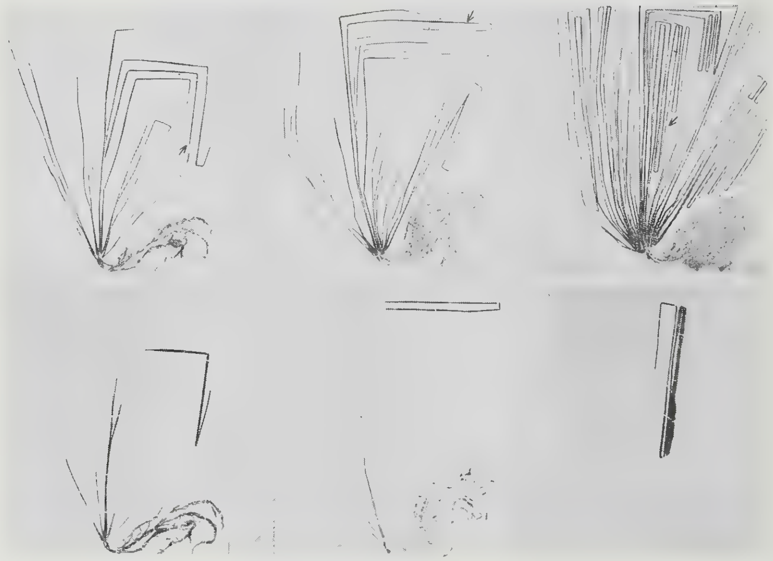
not reveal any significant autoradiographic artefacts which could have influenced the interpretation of the other autoradiographs.

The labelling pattern of plants at the one, two and four-tiller stages (Figs. 3, 4 and 5 respectively) were similar. Carbon-14 assimilates were exported mainly to young developing leaves of the main and daughter tillers and to the roots. In almost all other leaves there was a faint trace of radiocarbon.

A change in the distribution pattern of assimilates occurred at the six-tiller stage of development (Fig. 6). Export of ^{14}C-assimilates to the daughter tillers decreased considerably, although the roots continued to accumulate radiocarbon.

At the eight-tiller stage there was a similar pattern of distribution of ^{14}C-assimilates as at the six-tiller stage.

Export of labelled assimilates from the treated leaf decreased even more at the mature vegetative stage (Fig. 7). Only the roots received assimilates.



5 6 7

Figs. 5–7

Photographs of mounted plants above, autoradiographs below. Youngest fully expanded leaf on main tiller of plants at the four-tiller stage (Fig. 5), six-tiller stage (Fig. 6) and mature vegetative stage (Fig. 7), treated.

(b) *Treatment of oldest green leaf on main tiller:*

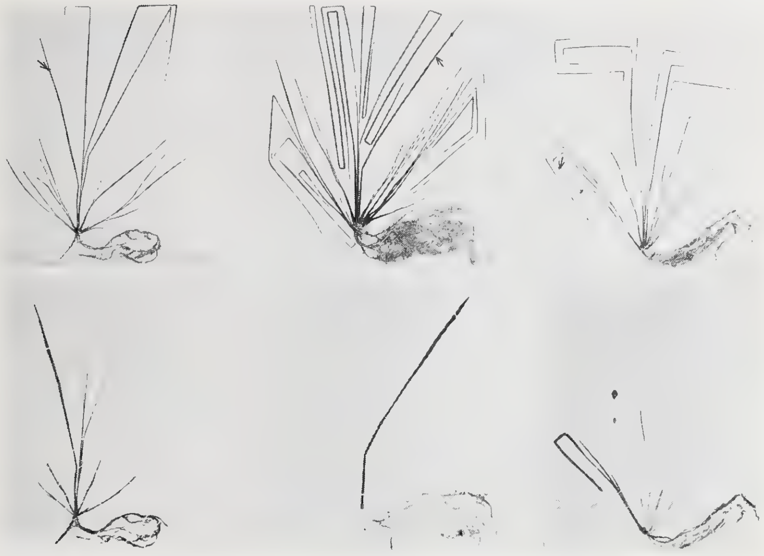
The patterns of distribution of labelled assimilates from the oldest green leaf on the main tiller, were very similar to those from the youngest fully expanded leaf on the main tiller at all vegetative stages of growth, in which treatments were imposed. Only the results for the seedling stage (Fig. 2) and for the four and six-tiller stages (Figs. 8 and 9 respectively), are shown.

Again, ^{14}C-assimilates from the treated leaf were translocated predominantly to young developing leaves of the main and daughter tillers and to the roots in plants up to the four-tiller stage of development.

From the six-tiller stage to the mature vegetative stage export of assimilates to the daughter tillers again decreased considerably although the roots continued to accumulate radiocarbon.

(c) *Treatment of youngest fully expanded leaf on oldest daughter tiller:*

At the two and four-tiller stages labelled material was translocated mainly to young developing leaves of the treated tillers, to the root systems and to a



8 9 10

Figs. 8–10

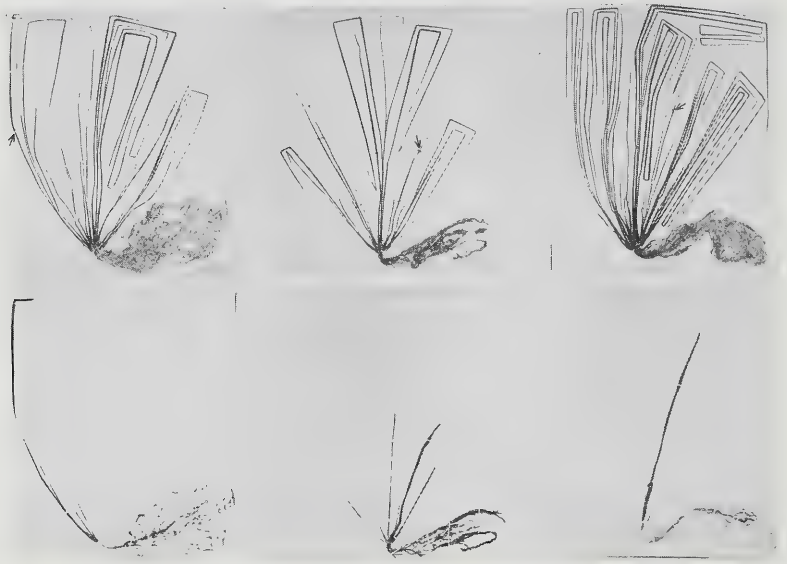
Photographs of mounted plants above, autoradiographs below. Oldest green leaf on main tiller of plants at the four-tiller stage (Fig. 8) and six-tiller stage (Fig. 9), treated. Youngest fully expanded leaf on oldest daughter tiller of a plant at the four-tiller stage (Fig. 10), treated.

lesser extent to growing regions of the main and other daughter tillers. Traces of radioactivity were present in most mature leaves. An autoradiograph of a plant at the four-tiller stage (Fig. 10) only is shown.

Again a change in the distribution pattern of assimilates occurred from the six-tiller stage to the mature vegetative stage. Carbon-14 assimilates from the treated leaves of plants at the six- and eight-tiller stages and the mature vegetative stage, were translocated mainly to growing regions of the treated tillers, to developing leaves of young tillers nearest them and to the roots. It is interesting to note that no assimilates were exported to the main tillers and translocation was restricted only to tillers on the same side as the treated tillers. An autoradiograph of a plant at the six-tiller stage (Fig. 11) only is shown.

(d) *Treatment of youngest fully expanded leaf on youngest daughter tiller:*

The distribution of labelled assimilates at the two and four-tiller stages, was similar to the distribution patterns that were obtained in plants at the same developmental stages, when the youngest fully expanded leaf on the oldest



Figs. 11–13

Photographs of mounted plants above, autoradiographs below. Youngest fully expanded leaf on oldest daughter tiller of a plant at the six-tiller stage (Fig. 11), treated. Youngest fully expanded leaf on youngest daughter tiller of plants at the four-tiller stage (Fig. 12) and eight-tiller stage (Fig. 13), treated.

daughter tiller, was treated. An autoradiograph of a plant at the four-tiller stage (Fig. 12) is shown.

In a plant at the eight-tiller stage of development (Fig. 13) almost all the assimilated carbon was retained by the treated leaf. Some labelled carbon was exported to young developing leaves of the treated tiller and to the roots.

Discussion

The patterns of distribution of labelled assimilates in *Eragrostis curvula* agree generally with findings in other members of the Gramineae in which similar investigations were carried out (Quinlan and Sagar, 1962; Williams, 1964; Sagar and Marshall, 1966; Marshall, 1967). All growing regions which are incapable of sustaining their own carbon requirements for rapid growth, make a demand upon the flow of assimilates from mature leaves. Assimilates, however, do not go indiscriminately to all growing regions. The stage of development and the position and age of the leaf that fixed ^{14}C, influenced the distribution patterns.

During the early stages of growth (the two and four-tiller stages) there was extensive movement of assimilates from the main tiller to the daughter tillers and vice versa. This reciprocal movement has also been reported by other workers. In wheat plants at the two-tiller stage, Quinlan and Sagar (1962), found that the main shoot passed labelled material to the two growing tillers. When these side tillers were treated, the reverse movement took place. Similar observations for reciprocal movement have also been recorded in young plants of *Lolium multiflorum* at the two- and three-tiller stages of development by Sagar and Marshall (1966) and Marshall (1967). Forde (1966a) also reported this phenomenon in *Cynodon dactylon* (L.) Pers. It appears therefore that reciprocal movement between tillers does take place in many grasses and that the stage of development is an important factor which governs this type of movement. This movement seems to be restricted mainly to the young stages of growth when there is a considerable interdependence of the various parts of a tillering grass plant.

During the later stages of growth, from the six-tiller to the mature vegetative stages, intertiller translocation decreased markedly. Although the main tiller continued to supply small amounts of assimilates to growing regions of young tillers closely associated with it, the movement of assimilates from the daughter tillers ceased. Translocation from the daughter tillers was restricted mainly to their own growing regions and to young tillers closely associated with and on the same side as the treated tiller. It appears therefore that in *E. curvula* tillers become increasingly independent from the six-tiller stage onwards.

Tiller independence in other perennial grasses has been attained at about the seven-tiller stage in *Lolium perenne* (Forde, 1966b) and at the four-tiller stage in *L. multiflorum* (Sagar and Marshall, 1966; Marshall, 1967). It is interesting to note that there was extensive intertiller translocation in *E. curvula* at the four-tiller stage. Williams (1964) noted that in *Phleum pratense*, movement of assimilates from one tiller to another did not occur if the tillers were large. Similar observations were made in rice by Shen (1960). Quinlan and Sagar (1962) found that tillers of spring wheat became independent after ear emergence, while in winter wheats, Lupton (1966) found that they became autotrophic earlier.

Williams (1964) suggested that tiller independence may be related to the stage of differentiation of the vascular strands. This led Forde (1966a) to conclude that tiller independence in *C. dactylon* was probably attained only when tillers formed their own nodal roots.

However, evidence for the free movement of ions between tillers throughout the plant, tends to contradict the apparent individuality of the tiller. Troughton (1960) and Marshall (1967) showed that ^{32}P was translocated to the whole of the plant after absorption by a single root of *L. perenne* and *L. multiflorum* respectively. Williams (1960) confirmed this for ^{35}S in *P. pratense.*

In addition, defoliation experiments have added to the controversy. Marshall and Sagar (1965) showed that partial defoliation of tillers in *L. multiflorum* could induce export of assimilates from a normally independent tiller to damaged tillers. These authors therefore suggested that the plant must be regarded physiologically as a whole, in spite of the apparent individuality of the tiller.

The conclusion that can be drawn from these findings is that tillers become independent, once they have developed sufficient leaf area for the supply of all the assimilates needed for their own growth, and have established efficient rooting systems for the absorption of water and mineral salts. Under conditions of stress, probably caused by defoliation or shortage of nutrients, a reintegration of independent tillers is possible.

In the present investigation, faint images of mature leaves appeared on many autoradiographs. Similar observations were made by Hale and Weaver (1962) in the grapevine. Quinlan and Sagar (1962) reported that in wheat, many mature leaves became labelled when a young leaf assimilated $^{14}CO_2$, and Hoshino, Nishimura and Okubo (1964) reported that there was some translocation into old leaves of Ladino clover when a newly opened leaf was treated.

These findings are in contrast to work carried out by other investigators. Thaine, Ovenden and Turner (1959) demonstrated that in soyabean, import into mature leaves was exceptional. Williams (1964) and Sanderson and Sivapalan (1966) observed no significant movement into mature leaves of *P. pratense* and tea plants respectively.

The possibility that the labelling of mature leaves in wheat occurred via the transpiration stream following migration of label from phloem to xylem, as in the case of translocated herbicides, was investigated by Quinlan and Sagar (1962). However, no indication of xylem transference was obtained. In the grapevine, Hale and Weaver (1962) noted that the activity in mature leaves was not present in the veins but was restricted to the intervenal region of the laminae. This observation suggested that radiocarbon probably reached the mature leaves in the gaseous form. Subsequent investigations by these authors, confirmed this. It is possible therefore, that a similar phenomenon was responsible for the labelling of mature leaves in *E. curvula* and probably also in wheat and in Ladino clover. Microautoradiographic evidence to be presented in another paper demonstrates that $^{14}CO_2$ may be distributed within the plant in the gaseous form.

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